



Targeted Protein Degradator Discovery Using the SPRINTer™ Cell-Based Assay Platform

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CHI Discovery On Target, Oct 19, 2022

Overview of Targeted Protein Degradation

Enzyme Fragment Complementation Assay Platform

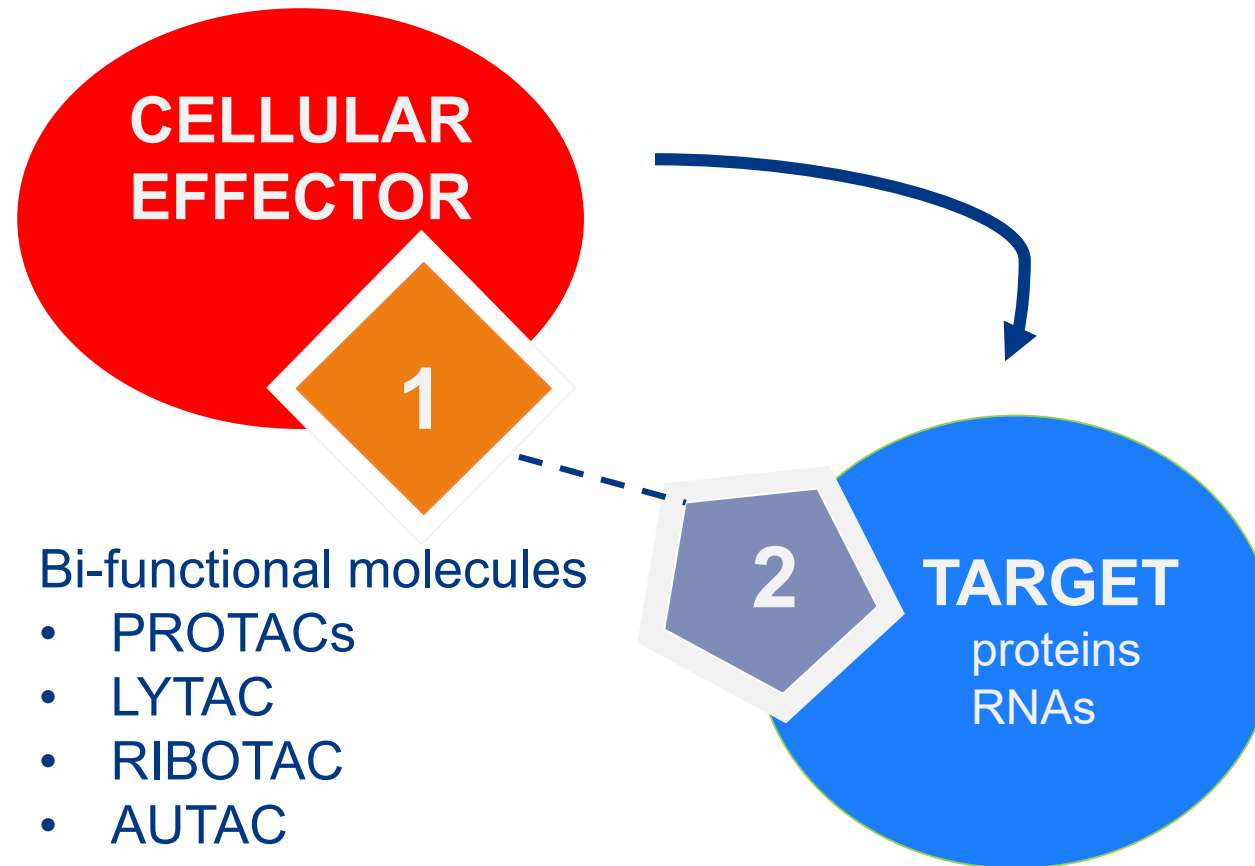
- SPRINTer™ Cell-Based Assays Platform
 - Protein degraders (BRD4, c-MYC, BTK), molecular glues, and E3 ligase inhibitors
- InCELL Target Engagement Assay Platform
 - InCELL Assay with SPRINTer Cell Lines
- Development of SPRINTer KRAS^{G12C} Assay

Conclusions

Targeted Protein Degradation

Cellular degradation systems

- Ubiquitin-proteasome
- Lysosome
- Autophage



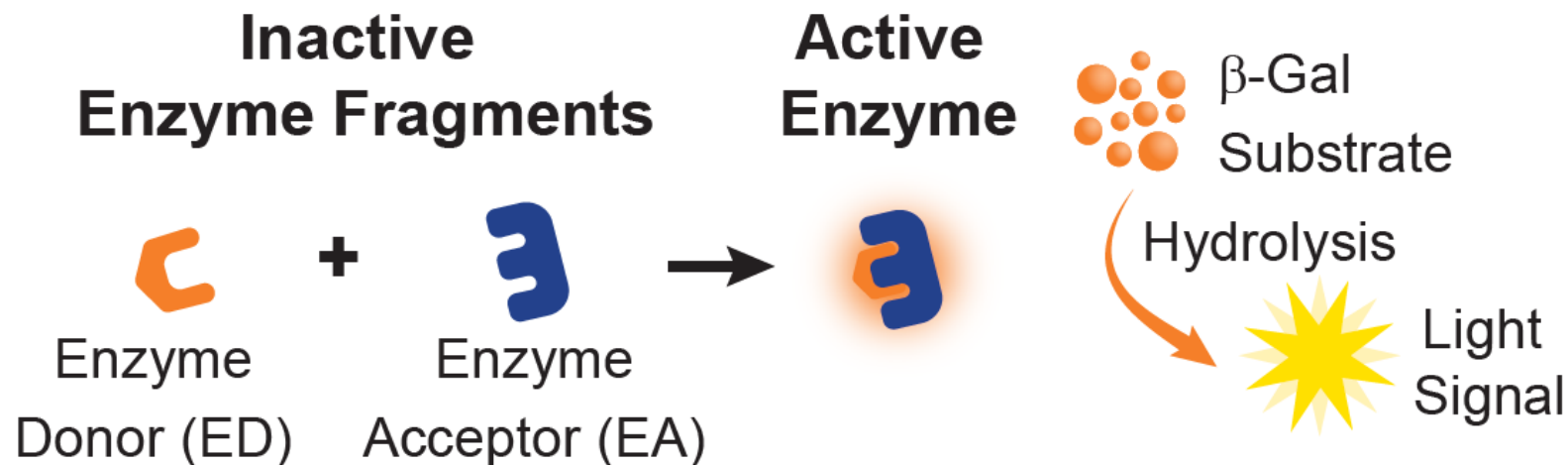
Bi-functional molecules

- PROTACs
- LYTAC
- RIBOTAC
- AUTAC

Need rapid, reproducible, and homogeneous cell-based assay amenable for high throughput

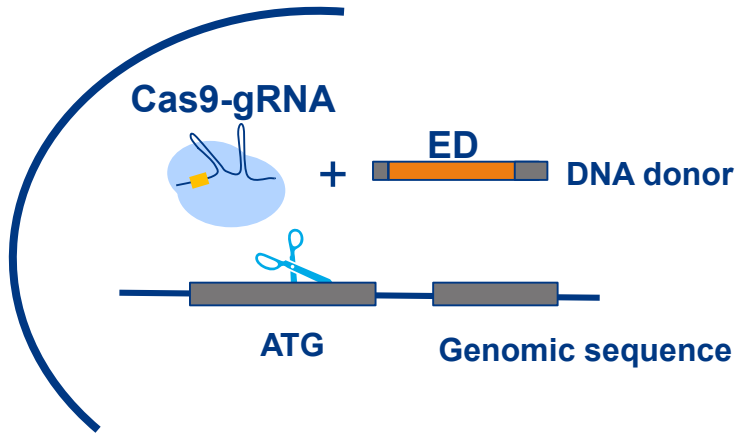
Enzyme Fragment Complementation (EFC)

- Robust and flexible platform that is well-accepted in the industry
- Flexible platform based on a split β -galactosidase enzyme
- Basis of Eurofins DiscoverX[®] cell-based functional and binding assays for multiple applications
- Combines fM reporter sensitivity with ease of implementation
- Simple, rapid, and homogeneous assay format amenable to high throughput

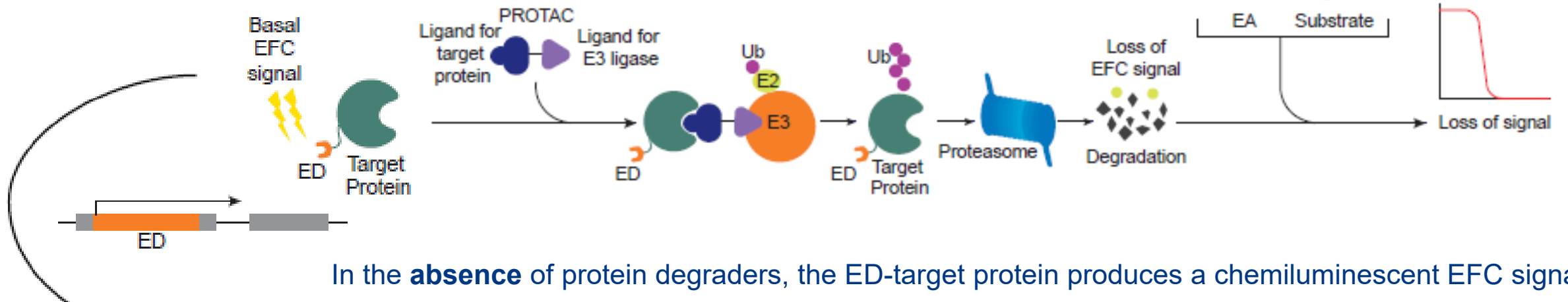


SPRINTer™ Targeted Protein Degradation Assays

Employing CRISPR/Cas9 and the Eurofins DiscoverX® EFC technology



Using CRISPR/Cas9 technology to generate a target protein tagged with a small EFC β -galactosidase enzyme donor (ED) fragment



In the **absence** of protein degraders, the ED-target protein produces a chemiluminescent EFC signal

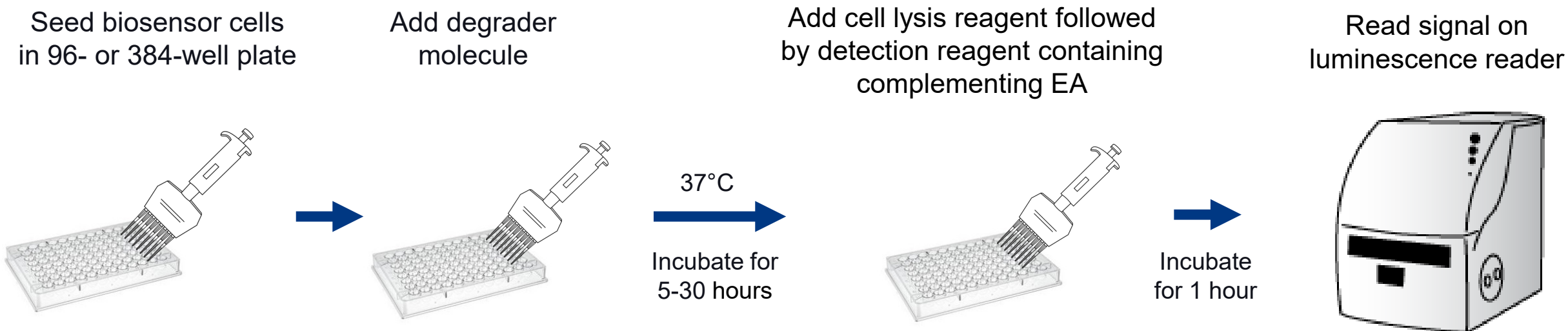
In the **presence** of protein degraders (e.g. **PROTAC**), the ED-target protein is subsequently degraded, resulting in loss of EFC luminescent signal

ED (Enzyme Donor), EA (Enzyme Acceptor)

SPRINTer assays are sensitive and scalable for detection of protein turnover

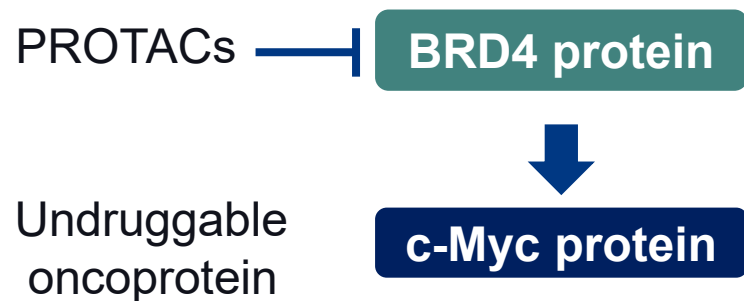
- Compatible with disease cell models expressing physiologically relevant levels of the target protein and relevant E3 ligase(s)
- Robust, reproducible, and easy to implement
- Easy-to-run, rapid, and homogeneous assay workflow amendable for high-throughput analysis
 - Rapidly detect intracellular degradation events in ~5 hours with simple assay workflow

SPRINTer Targeted Protein Degradation Assay Workflow



Easily Analyze a Variety of Targets for Protein Degradation

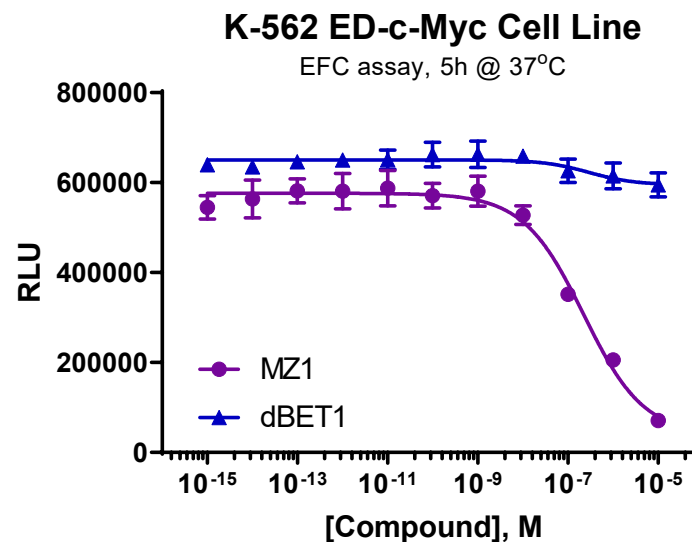
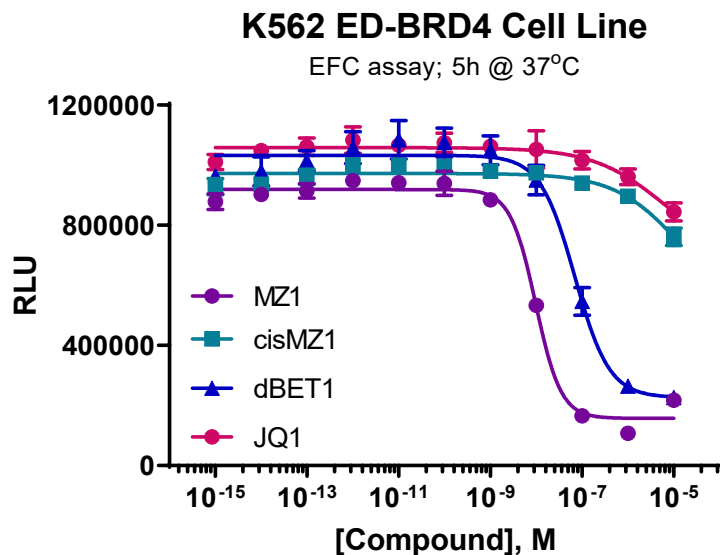
SPRINTer™ Assays: Analyzing BRD4 and c-Myc Degradation



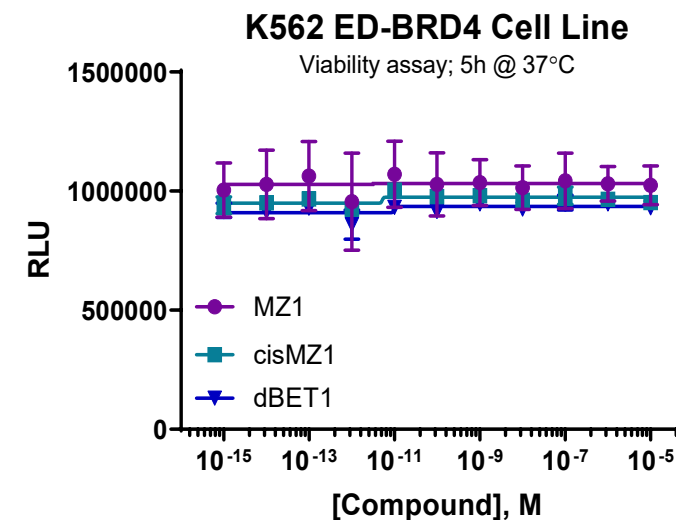
PROTAC Tool Molecules

Tool Molecules	BRD4 Ligand	E3 Ligase Ligand
MZ1	JQ1	VHL-1 (VHL)
dBET1	JQ1	thalidomide (cereblon)
cisMZ1	JQ1	--

EFC Assay (Functional)



Phenotypic Assay (Cell Viability)

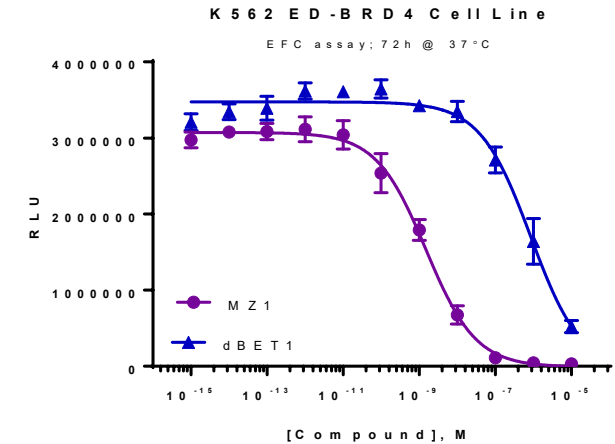
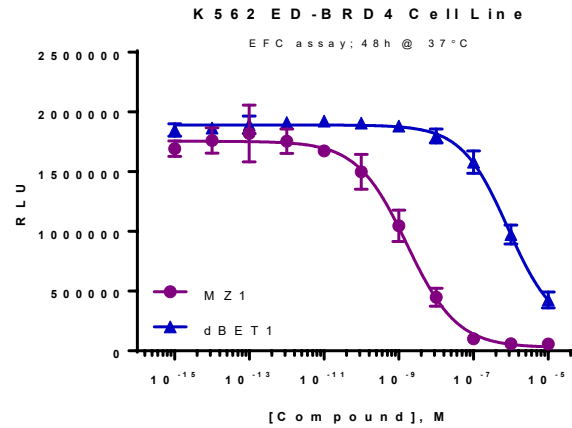
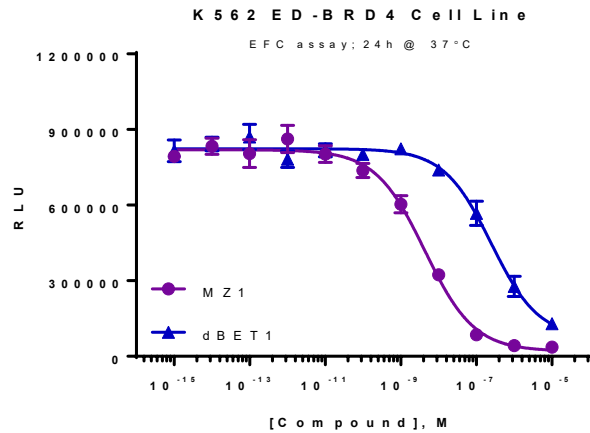


BET = Bromodomain & Extra-Terminal Motif; BRD4 = Bromodomain-containing protein 4

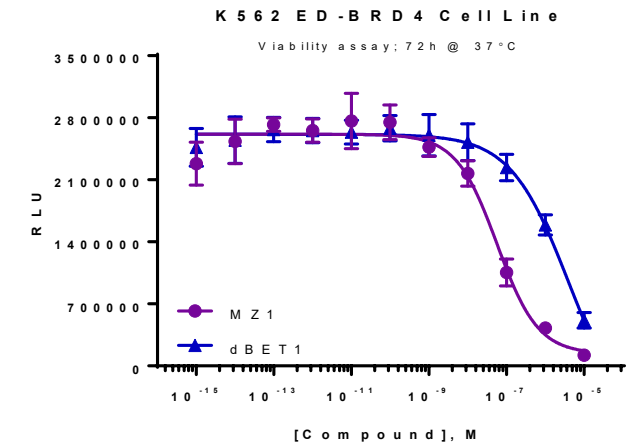
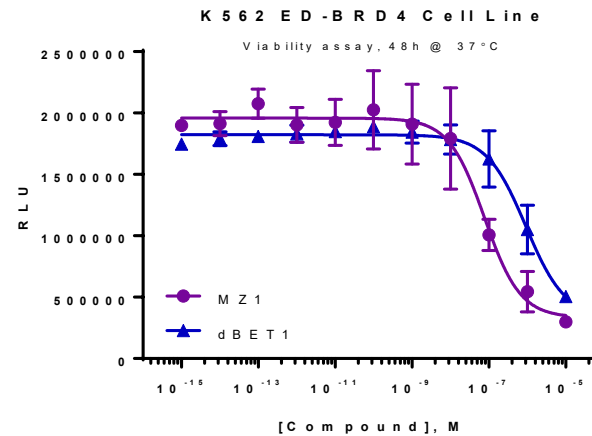
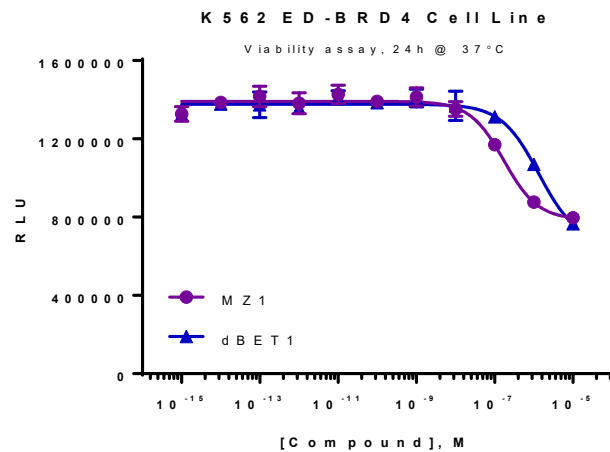
Evaluating the Time Course of Endogenous Protein Degradation Using SPRINTer™

Time course for PROTACs (MZ1 and dBET1) mediated degradation of BRD4

EFC Functional Assays (24, 48 & 72 hours)



Phenotypic Cell Viability Assays (24, 48 & 72 hours)



Evaluating the Time Course of Endogenous Protein Degradation Using SPRINTer™

PROTAC MZ1 mediated degradation of BRD4 and turnover of c-Myc in K562 cells

EFC Functional Cell-Based Assays

Biosensor	ED-BRD4 (K562)		ED-c-Myc (K562)		Fold Difference
	EC ₅₀ , nM	S/B	EC ₅₀ , nM	S/B	EC ₅₀ , nM
5h	9.7	5.8	222	15	22.9
24h	4.3	18	51	14.3	11.9
48h	1.6	28	27	32	16.9
72h	1.5	66	13	14	8.7

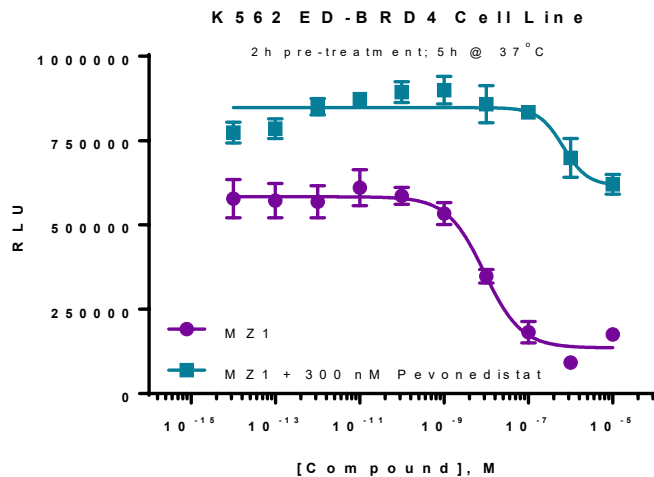
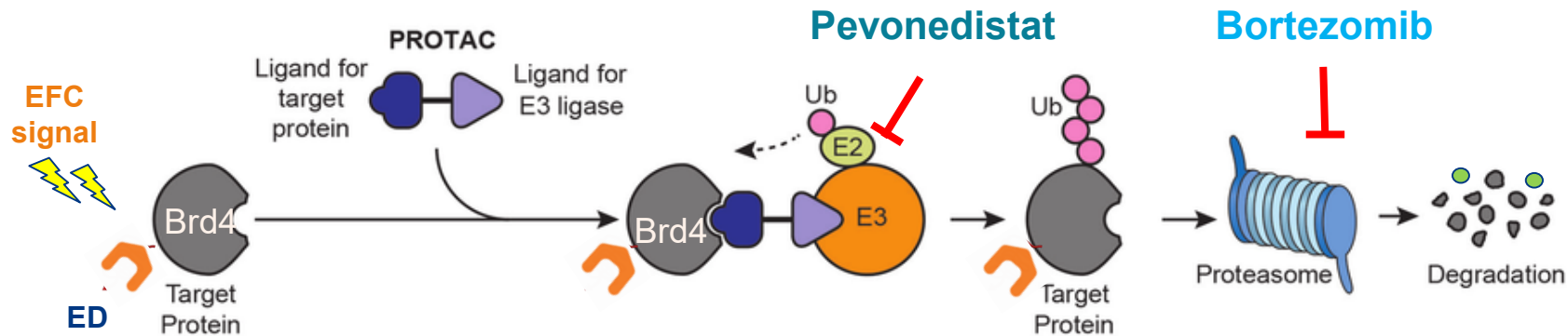
Phenotypic Cell Viability Assays

ED-BRD4 (K562)	
EC ₅₀ , nM	S/B
--	--
172	1.6
77	6.3
57	19

SPRINTer's EFC-based biosensor cells are fast and sensitive

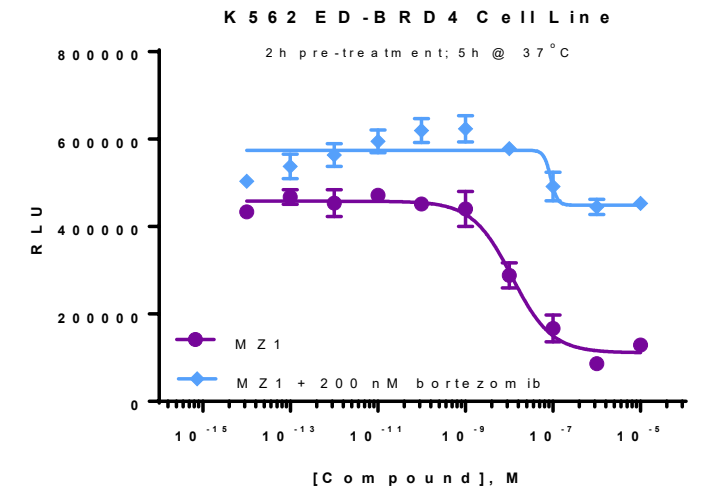
Characterization of SPRINTer™ Assays for Targeted Protein Degradation

Inhibitors of the ubiquitin-proteasome system block PROTAC-mediated target degradation



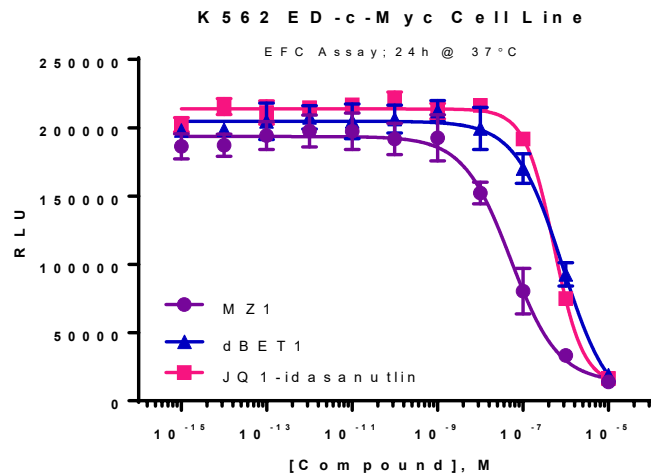
Sample	S/B	EC ₅₀ , nM
MZ1	6.3	9.0
MZ1 + pevonedistat	1.4	668

Sample	S/B	EC ₅₀ , nM
MZ1	5.4	11.6
MZ1 + bortezomib	1.3	89

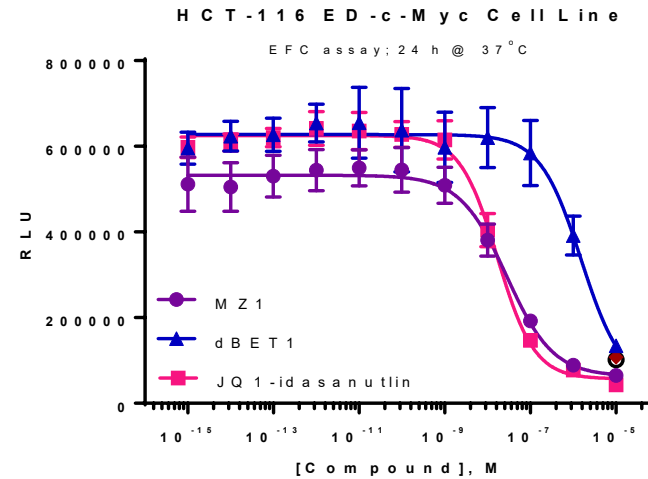


Characterization of SPRINTer™ Assays for Targeted Protein Degradation

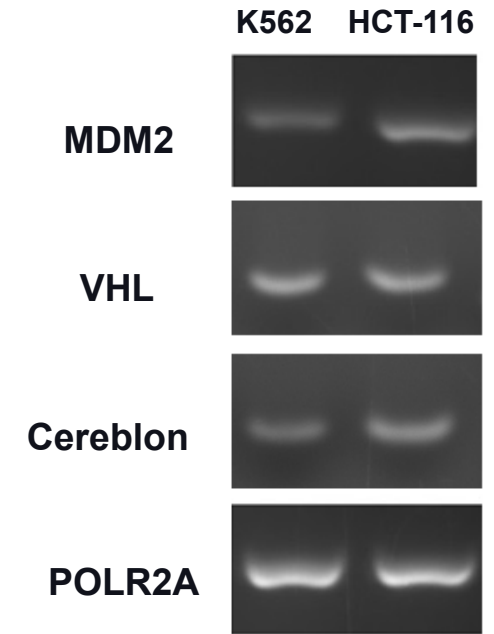
Differential PROTAC efficacies among cell models correlates with relative E3 ligase expression



MZ1 > dBET1 > JQ1-idasanutlin



MZ1 > JQ1-idasanutlin > dBET1



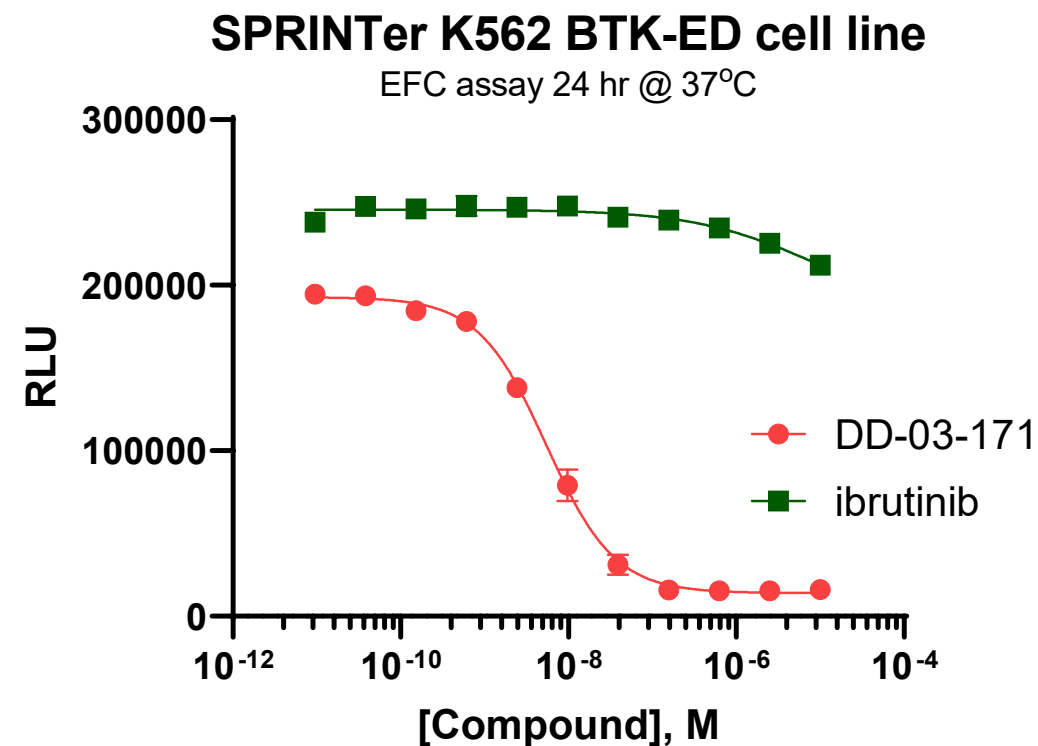
Relevant cell model makes a difference

PROTAC	BRD4 Ligand	E3 Ligase Ligand
JQ1-idasanutlin	JQ1	idasanutlin (MDM2)
MZ1	JQ1	VHL-1 (VHL)
dBET1	JQ1	thalidomide (cereblon)

SPRINTer BTK tyrosine kinase to identify degraders

Tool Molecules

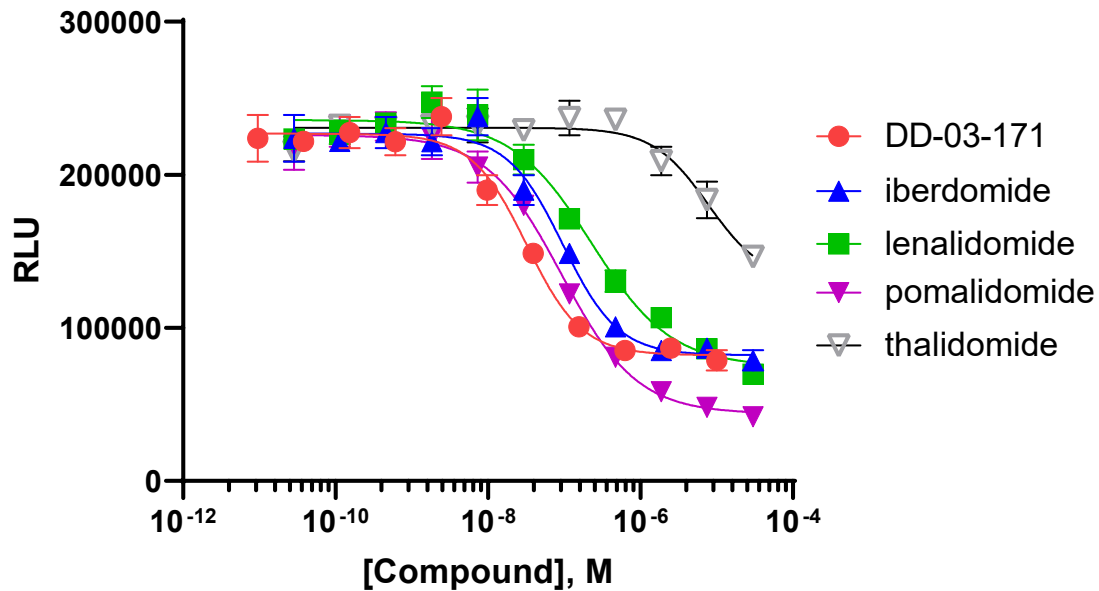
Tool Molecules	BTK Ligand	E3 Ligase Ligand
ibrutinib	ibrutinib	-
DD-03-171	CG1746	thalidomide (cereblon)



For molecular glues that target IKZF1 and IKZF3 (Aiolos)

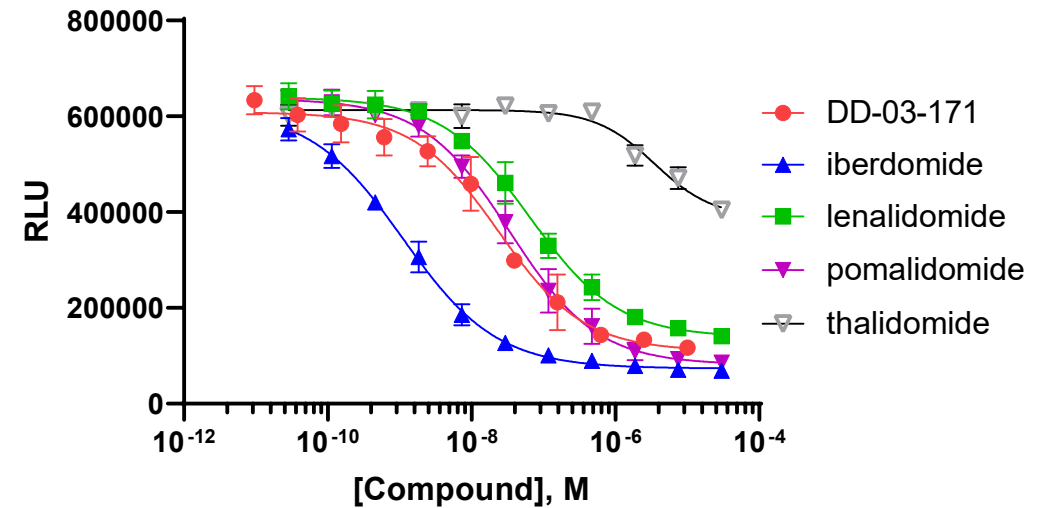
SPRINTer K562 ED-IKZF1

EFC Assay, 24h @37°C



PathHunter Hek IKZF3

EFC assay, 24h @37°C



A heterologous overexpression cell line

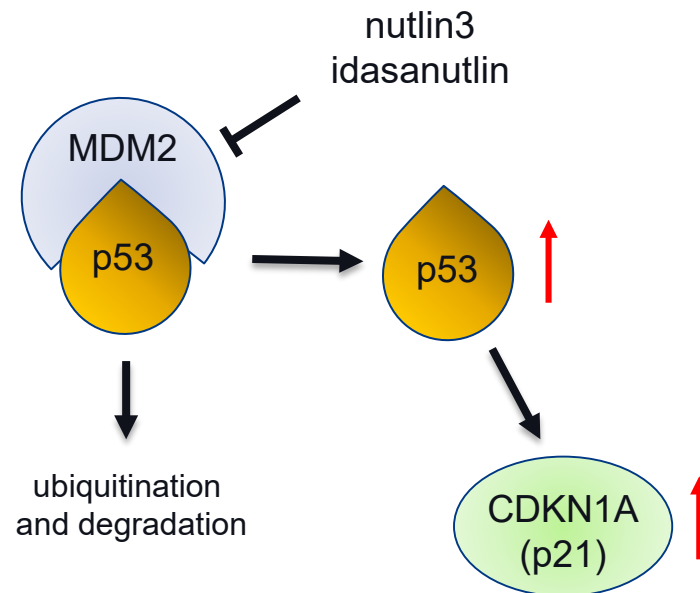
DD-03-171 > iberdomide = pomalidomide > lenalidomide >> thalidomide

iberdomide >> DD-03-171 = pomalidomide >= lenalidomide >> thalidomide

Tool Molecules

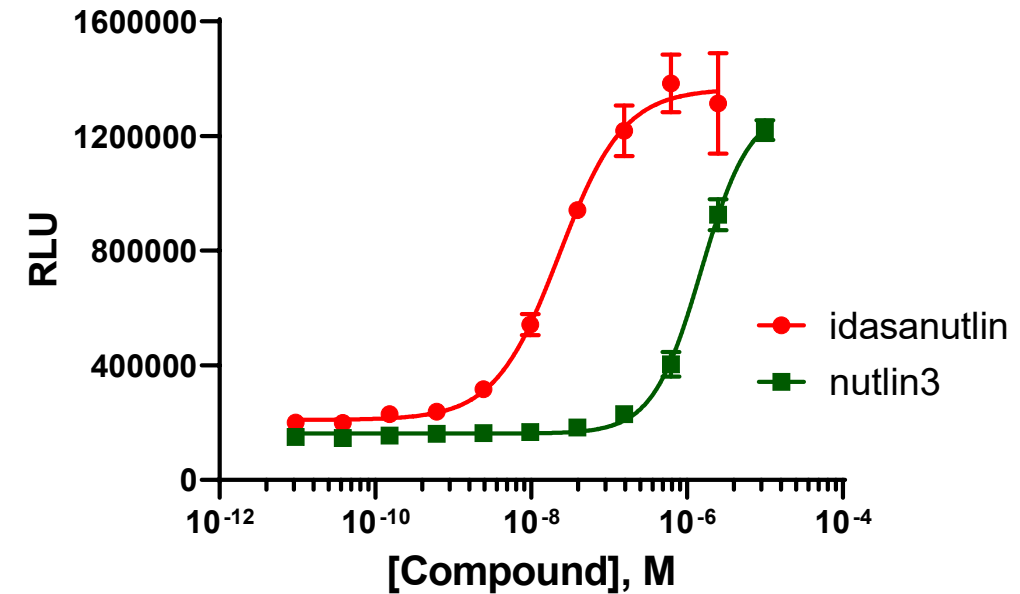
Tool Molecules	IKZF Ligand	E3 Ligase Ligand
DD-03-171	CG1746	thalidomide (cereblon)

E3 ligase inhibitors stabilize target proteins



HCT116 CDKN1A-ED (p21) Cell Line

EFC assay 18 hr @ 37°C



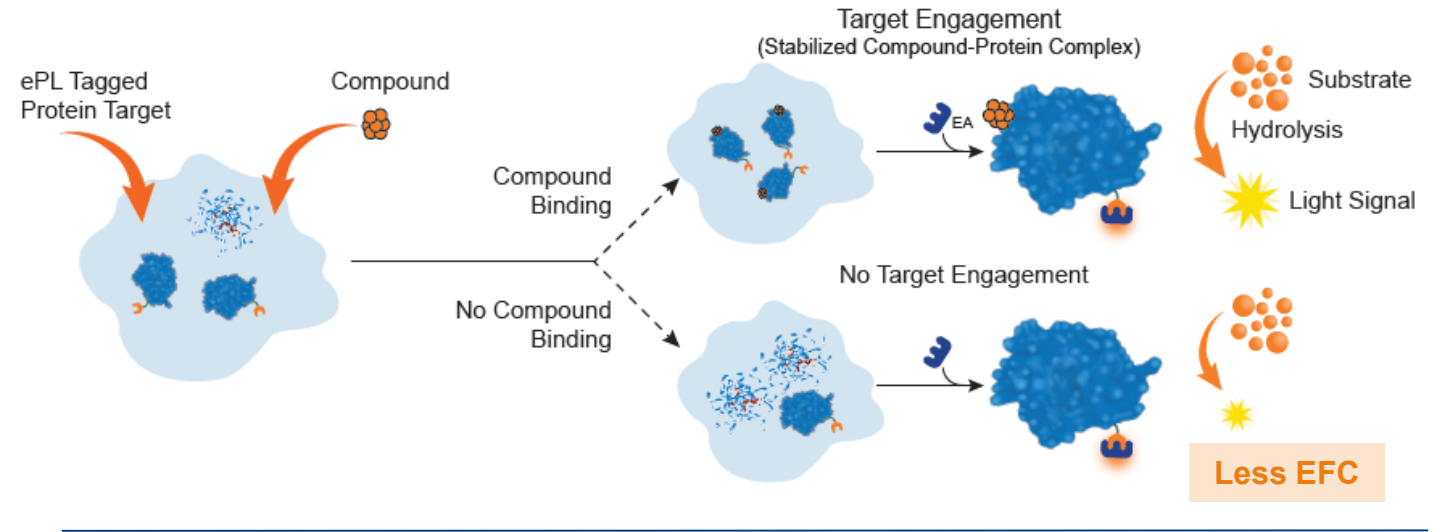
CDKN1A (p21) biosensor is a surrogate readout for TP53 protein stability

InCELL Compound-Target Engagement Assays

EFC-Based Assays

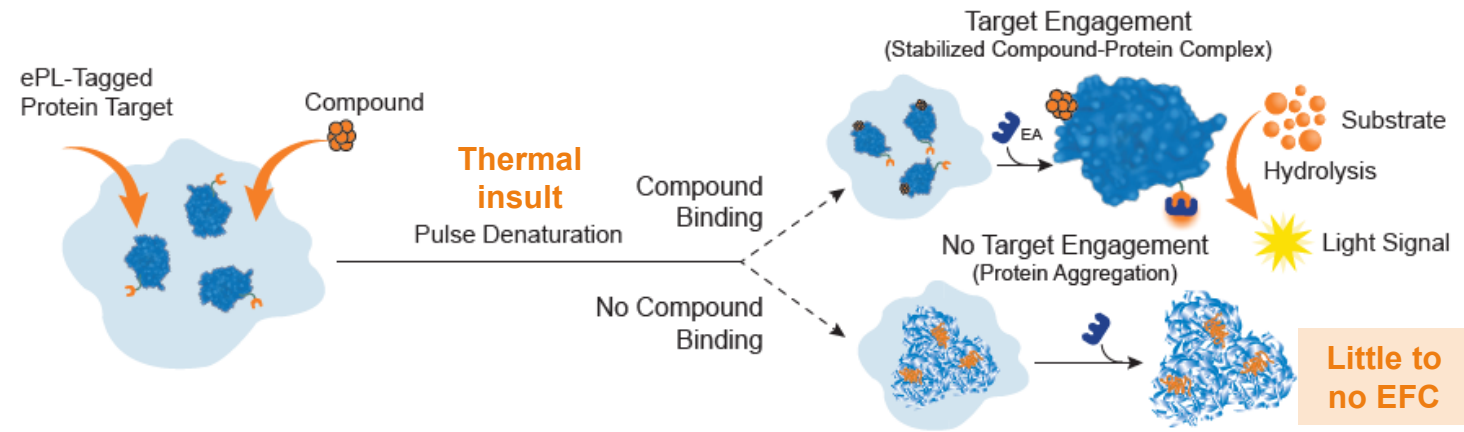
InCELL Hunter™

- Compound-target interaction to protect protein from cellular degradation over time
 - Relatively short-life ED (ePL)-tagged domains of the desired protein are typically heterologously expressed and subjected to quick degradation cellular machinery over time



InCELL Pulse™

Uses heat pulse to induce cellular protein turnover



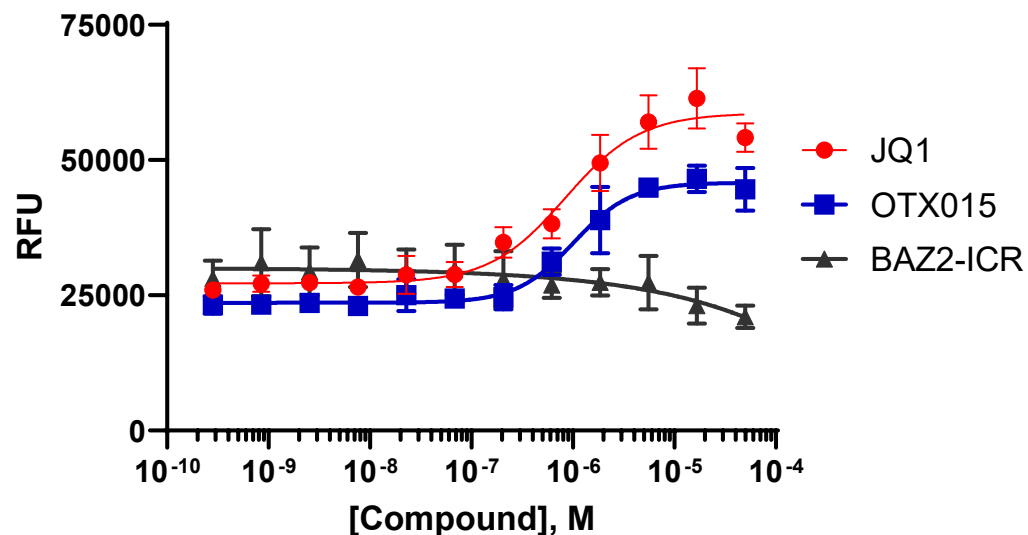
EFC (Enzyme Fragment Complementation), ePL = ED (Enzyme Donor), EA (Enzyme Acceptor)

InCELL Pulse™ Assay: SPRINTer BRD4 Cell Line

Endogenous full-length target protein
1h incubation with compound at 37°C; 49°C pulse for 3 min.

SPRINTer K562 BRD4 Cell (91-1002C042)

InCELL Pulse @ 49°C for 3 min



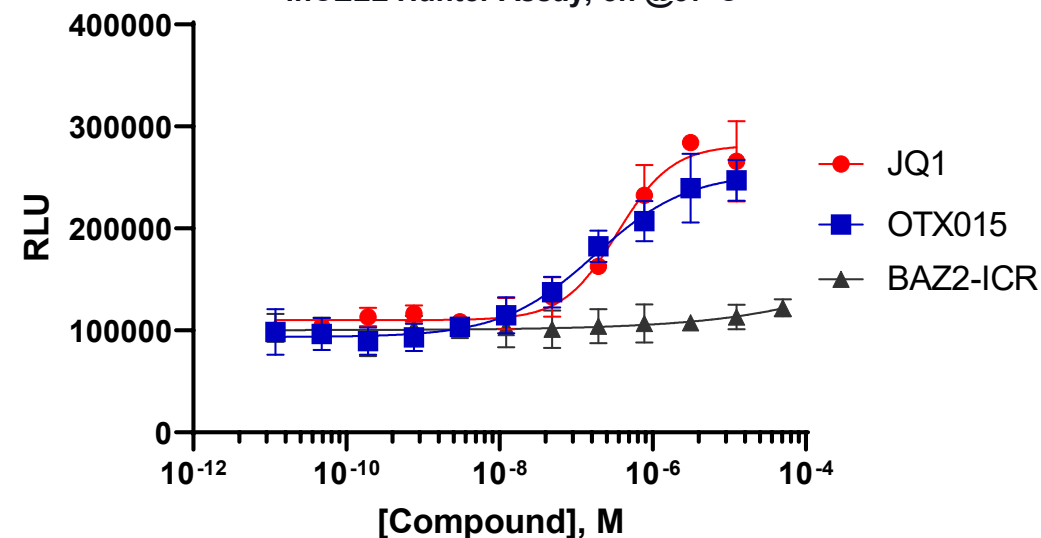
JQ1 > OTX015 > BAZ2-ICR

InCELL Hunter™ BRD4 Assay

Heterologous overexpression of BRD4(1) domain
5h incubation with compound at 37°C

InCELL Hunter HEK BRD4 Cell (96-0005C1)

InCELL Hunter Assay, 5h @37°C



JQ1 > OTX015 > BAZ2-ICR

Comparable rank order and assay window in both assay formats
SPRINTer format interrogates full-length protein

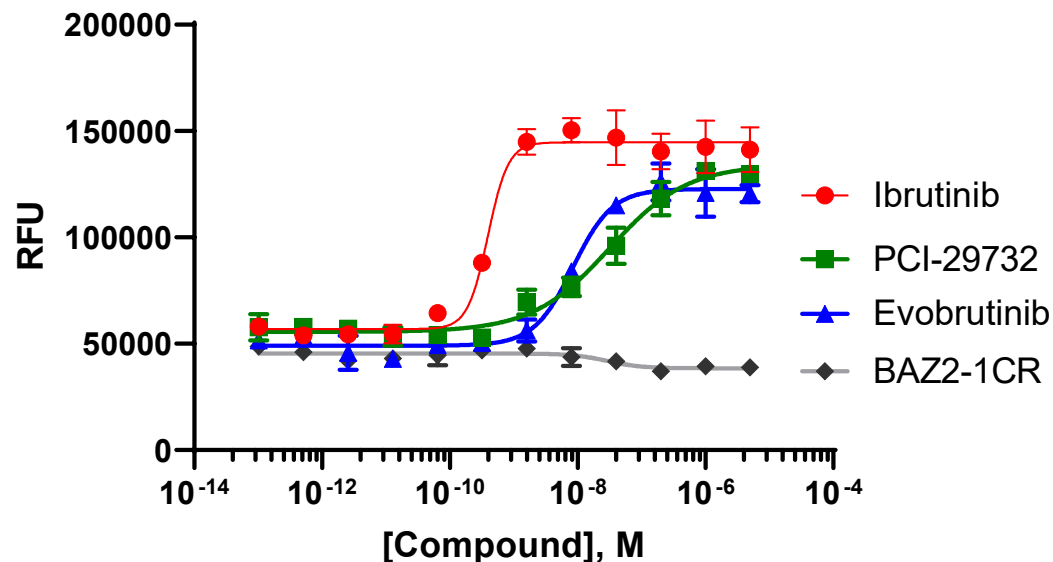
InCELL Pulse™ Assay: SPRINTer BTK Cell Line

Endogenous full-length target protein

1h incubation with compound 37°C, 45°C pulse for 3 min

SPRINTer K562 BTK (91-1005C042)

InCELL Pulse @ 45°C for 3 min.



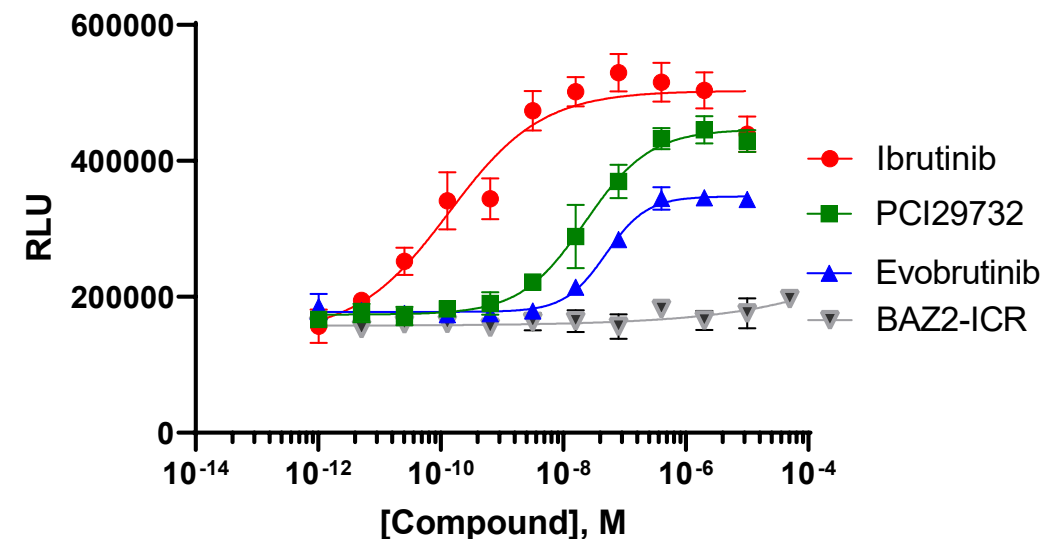
InCELL Hunter™ Assay

Heterologous overexpression of BTK kinase domain

3h incubation with compound at 37°C

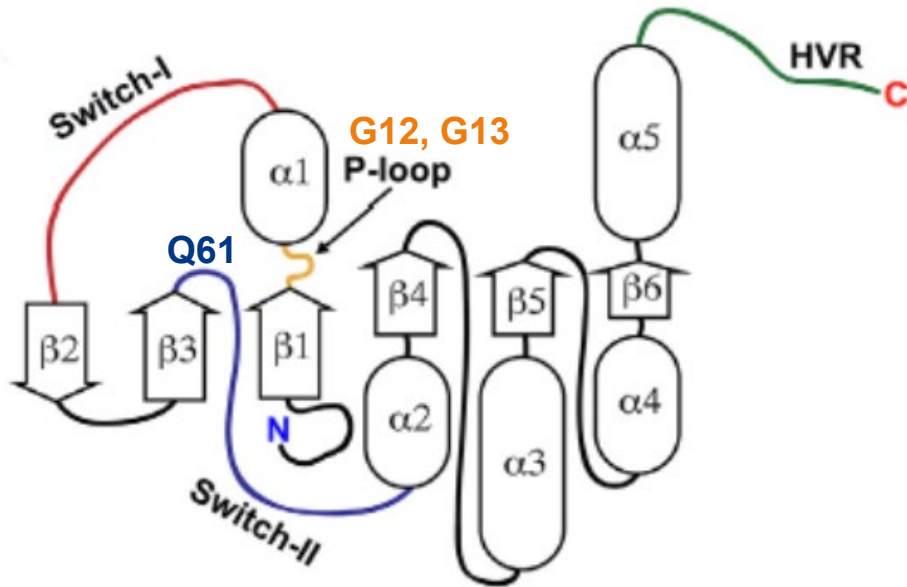
InCELL Hunter A549 BTK (96-0056C15)

InCELL Hunter, 3h @ 37°C

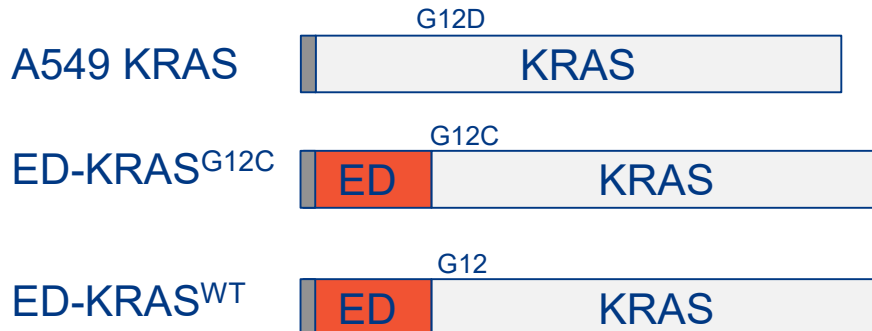


Comparable rank order and assay window in both assay formats
SPRINTer format interrogates full-length protein

Development of KRAS^{G12C} SPRINTer™ Assay



Pantsar 2020 *Comput Struct Biotechnol J*

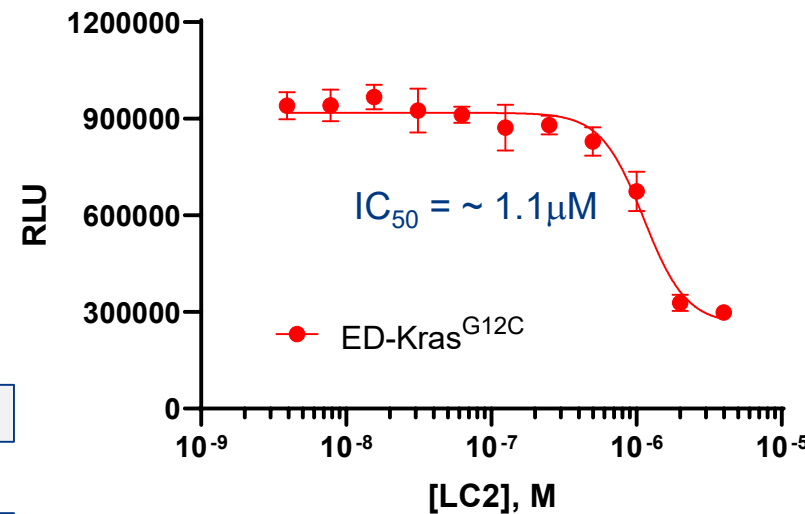


Functional Assay for PROTACs

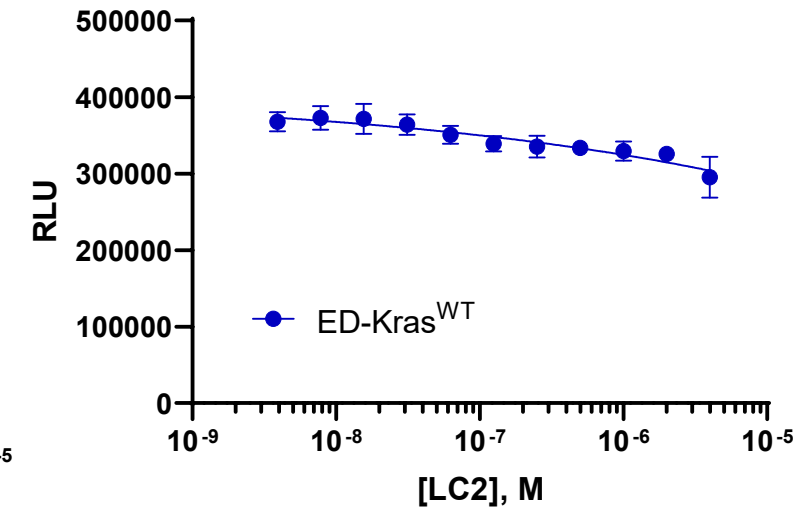
Tool Molecules

Tool Molecules	KRAS Ligand	E3 Ligase Ligand
LC2	MRTX849	VHL1 (VHL)

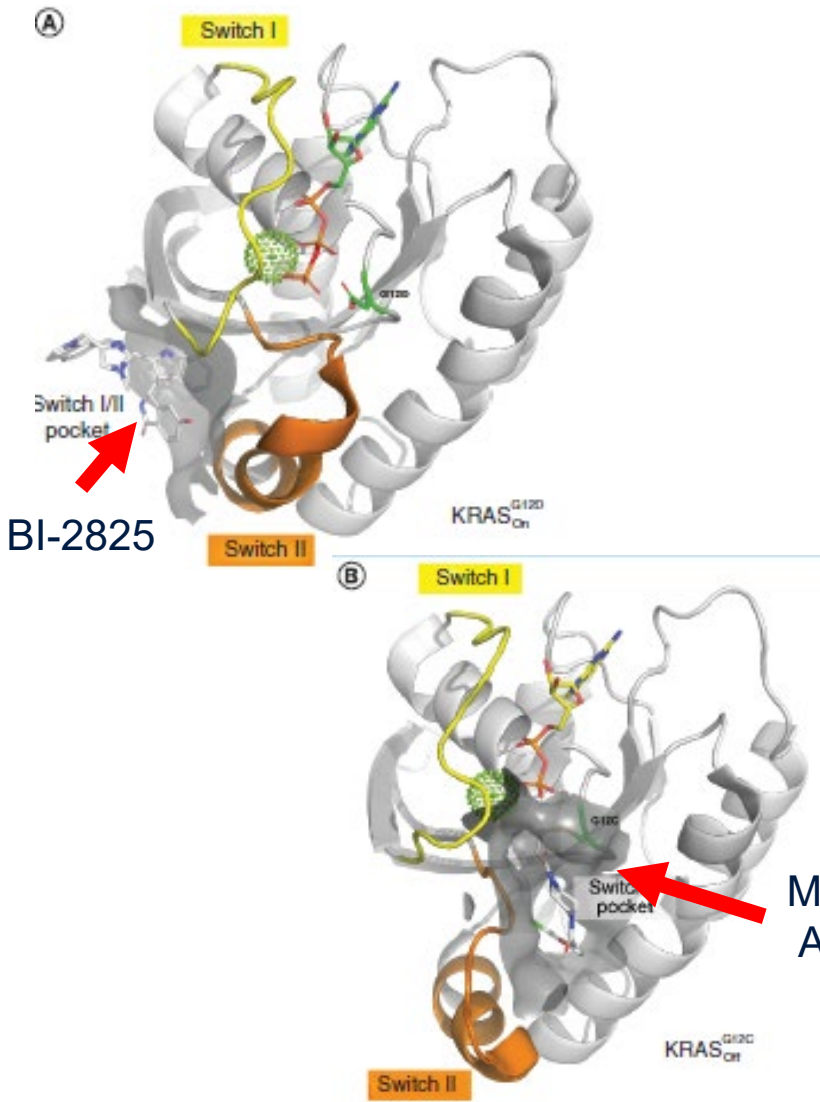
SPRINTer A549 ED-KRAS^{G12C} cell
EFC assay 18h @ 37°C



SPRINTer A549 KRAS^{WT} cell
EFC assay, 18h @ 37°C

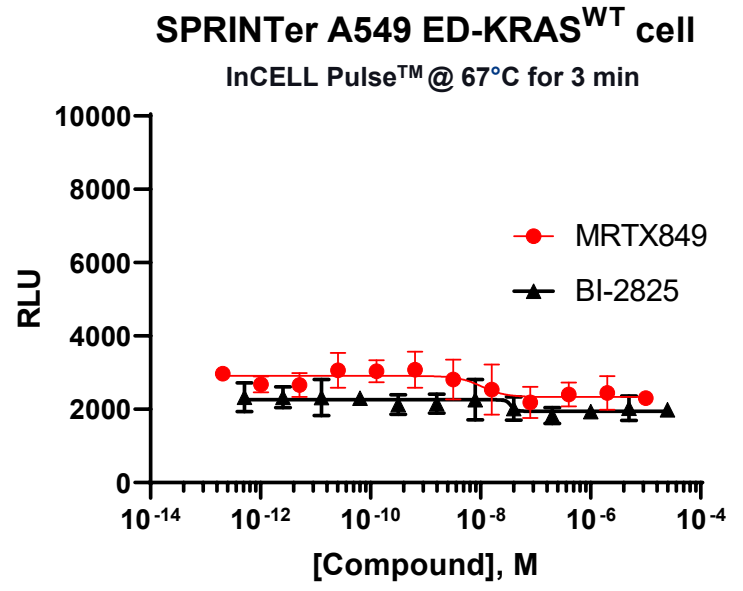
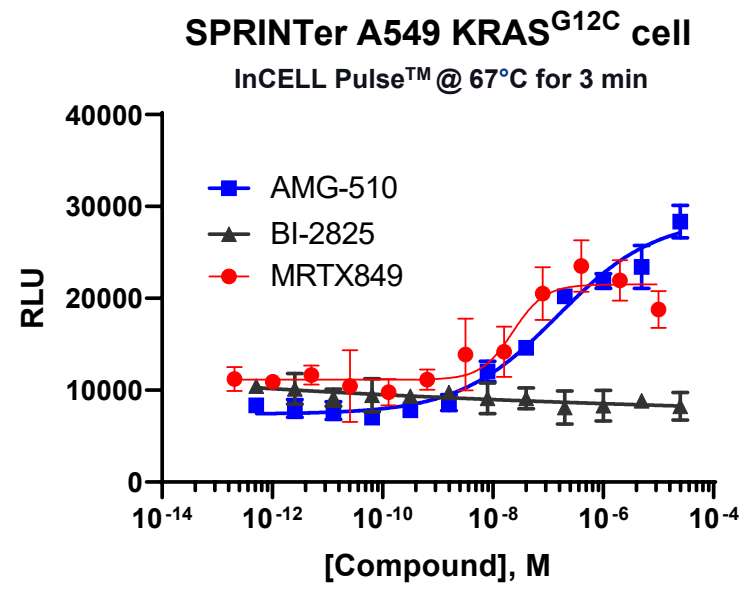


Evaluation of Target Engagement by KRAS^{G12C} Inhibitor in SPRINTer™ Cell Line – Detects Binding of Covalent Inhibitors



Tool Molecules	KRAS Target	KRAS mutations	Note
MRTX849	Switch II pocket	G12C	Covalent inhibitor
AMG501	Switch II pocket	G12C	Covalent inhibitor
BI-2825	Switch I/II pocket	G12D > WT	Non-Covalent inhibitor

Target Engagement Assay (ICP)



Kessler et al. 2020 Future Med Chem

SPRINTer™ assays are used to quantify protein turnover

- Screening platform to identify new molecular entities that modulate oncogenic protein levels for therapeutic development
- Medium-to-high throughput assays to quantify changes in endogenous protein levels in disease-relevant cell models
- Evaluate target engagement of small molecule inhibitors against a full length target protein
 - Suitable for rank ordering of leads or rapid evaluation of hits from a biochemical screen
- Off-the-shelf SPRINTer cell lines for several target (BRD4, c-Myc, BTK, CDKN1A, IKZF1) and more under development including KRAS^{G12C}



Learn more at discoverx.com/turnover